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STATUS REPORT - (NASA RESEARCH GRANT NsG-476)

[redacted]

Grant Title: (Primitive Earth Synthesis of Amino Acids and Polypeptides)

[redacted]

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During this period, two aspects of the problem have been investigated in detail.

1.) We have attempted to determine whether a heavy metal contaminant in ammonium cyanide contributes significantly to the formation of amino acids and amino acid conglomerates under the influence of heat. Reaction products resulting from interaction between gaseous HCN with aqueous ammonia and liquid HCN with aqueous ammonia have been compared. In essence, the reactions appear to progress to largely similar conclusions. The types of polymers, as indicated by concomitant examination in the automatic amino acid analyzer, indicate the formation of similar polymers. Analyses of hydrolysates of these polymers indicate the presence of the following amino acids in both types of preparations: aspartic acid, threonine, serine, glutamic acid, glycine, alanine, beta-alanine, iso-leucine and leucine, and amino acids with the elution time but obviously distinct from methionine and cystine.

2.) We have attempted to isolate sufficient quantities of three principal polymers formed, by combining continuous-flow electrophoresis and paper-strip chromatography. The results of this work are summarized in the enclosed abstract which has been submitted for presentation at the meeting of the Federation of American Biological Societies in April, 1964.

The direction that research will take in the coming six months appears to be as follows. We hope to isolate and purify sufficient quantities of several amino acid polymers for definitive molecular weight determination. If the molecular weights are of the order of 1000 or 2000, it may be possible to use depression of freezing point to estimate size. If the molecular weights appear to be larger, of the order of 10,000 or 12,000, it may be possible to use velocity analytical ultracentrifugal analysis. The resolution and preparation of polymers is being conducted largely by use of continuous-flow electrophoresis in the Brinkmann apparatus.

If sufficient polymers are isolated, we plan to examine the hydrolytic activity of proteolytic enzymes. This will probably be accomplished by adding arginine and/or lysine prior to interaction of aqueous ammonium and HCN since we know that the polymers formed will incorporate these amino acids. Trypsin appears to be the enzyme of choice under these circumstances. Enzymatic cleavage will be

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followed in a recording titrimeter. Cleavage product will be examined for amino acid constituents. The purpose of this program is largely to demonstrate the presence of a peptide bond.

We hope to isolate sufficient quantities of individual amino acids to examine optical activity. To date, it has not been possible to accomplish this because yields of amino acids have been too small. With the continuous-flow electrophoresis apparatus, it may be possible to obtain relatively large amounts of uncontaminated amino acids.

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INCORPORATION OF GLUTAMIC ACID INTO AMINO ACID COMPLEXES IN AQUEOUS NH_4CN. C. U. Lowe, Dept. Ped., State Univ. of N. Y. at Buffalo, Sch. of Med., Buffalo, N. Y.

Uniformly labelled C_{14} glutamic acid (40 uc, 28 ug) was heated 18 hours at 90° in 1 L 1.5 M NH_4CN. After centrifugation to remove black polymer, supernatant was passed through a charcoal column. Effluent contained 25% of radioactivity. Continuous flow electrophoresis in pH 2.3 HAc resolved two radioactive peaks: first proved by column chromatography to be 11.8% of original unreacted glutamic acid; second resolved by paper electrophoresis (HAc-pyridine, pH 6.8) into 20 ninhydrin reacting compounds. Three radioactive bands, identified by autoradiography, were eluted. Purity established by automatic amino acid analyzer in conjunction with a continuous flow beta scintillation detector. Reflux in 6N HCl, 24 hours, released following amino acids in ratios unique for each polymer: Glu, Asp, Gly, Ala, Ser, and a substance with elution time of lysine. Based on amino acid content minimal molecular weights estimated to be in excess of 5000. Laboratory synthesis of macromolecules containing amino acids may well mimic the production of paleo-proteins on the ancient earth. (Supported by NASA Grant NsG-476)